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IMMOBILIZED ORGANIC MATERIAL TO RELEASE A DEFINITE QUANTITY OF AGENT

[Immobilisiertes organisches Material mit

definiertem Wirkstoff - Release]

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/1[1]

The invention is related to new types of immobilization systems containing organic material allowing release of a definite quantity of reagent. Application of the new systems stated in this invention can be used for humans as well as inserted in the nerve tracts of organisms.

It is already known that patients are supported by extracorporeal bio-reactions (K.N. Matauma et. al. Surgery 101.1 pages 99 - 103 [1987]). Also described, is the transplantation with the help of microcapsules containing Langerhans' islands (A.M. Sun et. al. Trans Am. Soc. Artif. Intl. Organs 32, pages 39 - 41 [1986]). In the same way, bio-hybrid materials such as hollow organs with chambers for the inserted organ cells (EP 05 04 781 A1 23, 09, 92) have also been stated. The disadvantages of extracorporeal medication are seen in in-patient treatment as well as in the limited functioning of the biomaterial used. The major disadvantages of the transplantation are seen in the form of sedimentation and adhesion resulting in the transplants as well as the immune reactions of the host organism becoming ineffective. The specialty and the advantage of the system the invention talks about, is in the release of a definite quantity of agent. The release of the agent…[sic].

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[1] Numbers in the margin indicate pagination in the foreign text.

Description

As per latest estimates, of the 4 million diabetics, about 160,000 are of the type I. In their youth itself, they were advised multiple injections of insulin everyday. Even those falling under type 2 originally not prescribed insulin injections have been requiring insulin with prolonged illness. Even if the manifestation of diabetes is considered as the sole risk factor, it is enough to reduce the life expectancy by twenty one years in the case of a person who has been a type I diabetic for ten years, because the long-term diabetic syndrome affects multiple organs. The peripheral and autonomic neuropathy, retinopathy, micro- and macro-angiopathy of restricted functionality right up to diabetes requiring dialysis are of socio-political and economic significance. The appearance or the progression of long term diabetic syndrome can be avoided only by means of a normal or close to normal blood sugar count, as has once again been proven by the American DCCT - study.

The near-normal blood sugar count can be achieved only by means of an intensive insulin therapy but the hypoglycemic metabolic reactions must also be taken into account. The actual goal must therefore remain the same:

To achieve time- and requirement- related release of insulin.

Artificial endocrine pancreas (AEP): A purely technical alternative to this is the artificial endocrine pancreas (AEP), in which the glucose sensor based on the GOD method is coupled with an insulin pump. Interstitial measurement of the sugar content in the tissues and application of insulin in the subcutaneous tissues are the limits of the whole system. The first effect of insulin is seen only after 25 minutes so that any possibility of a closed loop system is ruled out. Since the effect of the so-called closed - loop system of physiological regulation can slow down the delayed organic damage, the purely technical solution has not been able to obtain a breakthrough yet. Normoglycemia with the objective of a timely and need - based release of insulin is possible only by a biological replacement of the insulin producing islet cell apparatus through the pancreas or by islet cell transplantation. Free allogenic transplants require intensive immunosuppression. This is why it could possible to develop "bio-artificial pancreas" successfully in a growing number of patients by a biological replacement of an organ i.e. by using isolated islet cells of a pig. In addition to this, the above mentioned immunosuppression can be prevented by using immuno-separation membranes.

Objective

Bio-artificial pancreas

Insulin producing cells that react to glucose stimulants by a timely or need-based release of insulin are implanted in a diabetic organism. The artificial membranes protect the free islet cells of allogenic or kenogenic origin from being destroyed by the immune system of the receiver. Two different forms of membranes based on diffusive soft transport that are being discussed presently.

1. Macro-capsulation in capillary membranes (Diagram 1):

   Here, the islets are capsulated into capillary membranes with diameters ranging from 0.5 to 4 mm and length between 2 and 3 cm and these are then implanted in the free abdominal cavity. This method, which was so far used only for experimentation on animals, has the advantage of exact reproducibility and characteristics even in the in - vitro model. The lower packing density of the islets in the capillary membrane proves to be a limiting factor as it results in a relatively larger volume to be implanted. The dilemma is that the most suitable compartment on the basis of volume i.e. the peritoneum is not considered following the danger of peritonitis.

Another form of macro-capsulation is the linking of "bio-artificial pancreas" to the blood circulation with the help of a vascular prosthesis. The blood flows into the capillary membranes and the islets in an artificial space created around the capillary membranes. This method is reported to have had only partial success (Diagram 2).

2. Micro - capsulation: In micro - capsulation, individual islets are enclosed in the smallest possible capsules made of alginate complexed with polylysine and transplanted. The microcapsules have a diameter of 0.5 mm. This technique is very popular because experiments on animals have shown that this technique increases the survival time of the transplant substantially in comparison to the non - capsulated islets in the control tests. The results however cannot be reproduced in a uniform manner as the capsulation technology so far has not been standardized (Diagram 1). The reasons for this are:
   1. The alginates are not standard extracts of brown algae with polyvalent cations, and similar to their affinity are more or less complexable. The alginates can set off foreign - body reactions on coming in contact with mitogenic factors after implantations.

2. The immuno-separation membrane is formed ion the presence of the tissue to be capsulated. The processes of membrane transformation take place under physiological conditions and hence cannot be controlled completely as is the case with the complexing process.
3. Most of the microcapsules of a diameter of half a millimeter have a volume several times larger than that of the islets of Langerhans, which are 50 - 300 μm in size. Narrower the membranes are to an islet, or the smaller a microcapsule is, the better they are for nourishment and insulin - release at the expected conditions. This also means that thin and closely fitting membranes can be implanted in other compartments with better oxygenation.

Before using a bio-artificial pancreas or other endocrine organ as therapeutics not only in connection with insulin-deficient diabetes but also with other endocrine deficiency syndromes, it is necessary that certain basic examinations be conducted.

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1. Definition and Control of the capsulation process and of the materials used.

2. Examination for optimization of microcapsule - membranes from the point of view of biocompatibility and immunology or immuno-separation.
3. Examination performed on grown animal
    a. Exact description of the glycometabolism (glucose metabolism) after transplantation.
    b. Evidence of the absence of a long - term diabetic syndrome.
    c. Evidence and functioning of the bio - artificial pancreas.

Discussion

From process of membrane building right up to adhesion the reason for the restricted clinical use of what has been said so far, is that in the case of all the models of examination discussed, the more and more restricted oxygenation or nutritive supply jeopardizes the effectiveness of the transplantation. Although the compartments such as peritoneum preferred so far, are suited for studying patho - morphogenetic principles, they are not suitable in the case of humans owing to the risk of peritonitis. Owing to the basic problems mentioned, Perinephric capsule or mesenterial fat tissues are a limitation to the success of this method.

In the vascularization model with a connection to the blood circulation, great importance is attached to the hemodynamic parameters that the plasma factors adhering to the membrane cannot unfold their functionally imparing effects by the corresponding flow conditions. To put it in another way, adhesion can be impaired only when a sufficient volume of flow in the compartment predominates or if the hemodynamic parameters guarantee sufficient oxygenation or nutritive supply, without having an adverse effect on the compartment itself. For these reasons, the endocardial region could be a suitable compartment. Similar to the transvenous access of a catheter fixed in the endocardial region, it is a case of an easily accessible compartment. Likewise, a problem - free exchange is guaranteed, when a flexible capsulation having the structure of a catheter is used with proximal or distal attachments.

This invention focuses on combating the inhibitive effects of blood constituents on the surface of the membrane. In this context, it is interesting to see that extravascular systems have the advantage of lower operative expenditure on device - constructions.

The impact of electrically conductive polymers on interactions between blood and blood constituents as well as the artificial surfaces correspond to the claim 5 of this patent.

The impacts of blood on artificial surfaces known so far, are as under:

1. Adsorption of proteins
2. Platelet reaction
3. Erythrocytes
4. Leucocytes
5. Compliment activation
6. Fibrinolytic activity
7. Objectives and variations in the test parameters

The following section describes specifically the membrane building process between blood and artificial surfaces and then follows it up with a discussion on the changes required in the micrometer range in order to influence the membrane - adhesive effect of plasmatic systems and molecules. The provisional catalogue towards the end outlines the scientific questions with regard to characterization of polymers, functional tests, and tests for biocompatibility.

To be able to make specific changes to the polymeric surface, it is absolutely essential to know all the possible interactions between blood plasma and artificial surfaces. When characterizing the systematic components such as platelet activation, intrinsic - extrinsic systems, overall stretch of coagulation and the control systems participating in the retardation of thrombus and

fibrinolysis in lingual terms, it is very easy to represent blood coagulation with the help of definite schematic terms (Diagram 3).

The processes mentioned, can be used to describe the interactions that take place when blood comes in contact with artificial surfaces. Two basic differences are seen in this regard:

1. An artificial surface of the membrane with any modification is not in a position to prevent activation of local coagulation and even the endothelial structures. Unlike endothelia, artificial surfaces are less suited to unfold any adhesive effects.
2. While under physiological conditions, the endothelium is not in a position to adsorb proteins, the adsorption of proteins is a major component involved in the interaction of blood with polymer.

Adsorption of proteins may be seen as the obligatory response of blood to an artificial surface. Likewise, the platelet adhesion is partly associated. This includes aggregation and release of factors, activation of the intrinsic pathway, cooperation of fibrinolysis of the complement and the Kallikrein - Kinin system as well as cellular elements. All this inevitably leads to the formation of thrombus as a result of which, during clinical application, a pharmacotherapy with anticoagulants, platelet

aggregation inhibitors, and plasminogen activators become necessary as simultaneous treatment.

1. Protein adsorption on artificial surfaces:

Protein adsorption on artificial surfaces is a spontaneous process during the blood - surface interaction. This adherence is of very critical importance, because it influences all the subsequent manifestations. The important role played by the adsorbed proteins is scientifically reflected in terms of the terminally conditioned coating. This coating however does not act passively. There is a possibility of a temporary adherence, denaturization, changes in conformity of the molecule as well as continuous change in the type of protein coat as well as in physical and biological properties. The property of proteins to spontaneously adhere to the artificial membranes is related to the amphipathic (dipolar ionic)

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character of the proteins that is to form polar groups in acidic or basic medium, and to be neutral to charge in the region of the isoelectric point (dielectric constants). (Electrophoretic mobility is 0 at the isoelectric point). Remark: Molecules that carry no charge in a salt - free solution are isoionic. This dipolar character of the proteins

develops the driving energy for the accumulation of concentration in the polymeric space. The low solubility of proteins is owing to the high molecular weight of the macroglobulin. When the reaction is exergonic, a reduction in the enthalpy results in the reduction of free energy of the system. The other possibility is an increase in the entropy resulting from a hydrolysis in the proteinaceous region or in the artificial surface. Type and extent of protein formation (protein attachment) decide the nature of the artificial surface. Adsorption on a glass surface is of the electrostatic nature. Adhesion and binding on polymeric surfaces is triggered off by hydrophobic surfaces, where the interaction between non-polar protein groups and non - polar artificial surfaces is achieved only by a polar aqueous medium. Generally, the binding on hydrophobic surfaces is stronger than that on hydrophilic surfaces. Hydrophobic properties of polymers also influence the steric conformation in adsorbed proteins. The adsorption on hydrophilic surfaces is more strongly and more quickly reversible when compared to that on hydrophobic surfaces. Reversibility is the state of equilibrium in binding. When blood makes contact with an artificial surface the proteins selectively accumulate forming a mono-layer film with a thickness of about 100nm, which in this form is a controlling function for the subsequent

interactions. From the point of view of the impact on blood platelets, the following proteins are most commonly examined: 1. albumin, 2. fibrinogen, 3. gamma - globulin (IgG). It is understood that after earlier adsorption of albumin, the subsequent adhesion of platelets does not take place but in the reverse case with primary adsorption of fibrinogen or gamma - globulin (IgG), adhesion of platelets is promoted.

It has been reported that adsorption of fibrinogen is preferred over that of albumin and gamma - globulin (IgG). This preference given to fibrinogen was also observed in the case of lipoproteins and other coagulation factors. Fibrinogen exerts a strong attractive force on the platelets and the reactivity of platelets correlates to the degree of preferred adsorption of fibrinogen, although the impact of proteins reduces after rapid changes in conformity. The close physiological relationship between fibrin and platelets is substantiated particularly by the fact that defibrinated or afibrinogenaemic plasma does not support the accumulation of platelets, till such time fibrinogen is added to the plasma again.

The presence of fibrinogen is necessary for the ADP - induced aggregation of platelets to get going (two classes of ADP - stimulated platelet receptors). It is then possible that the adsorption of fibrinogen on artificial surfaces does not proceed

independent of deposition of platelets, which reacts with specific receptors of the adhered platelets.

The temporary retention of fibrinogen adsorption is referred to as "Vromann Effect." The interchange of adsorbed fibrinogen with high molecular weight kininogen is a protein, which plays a decisive role in the contact activation of the intrinsic coagulation. For instance, a low "Vromann Effect" would imply that the intrinsic coagulation is less activated owing to fibrin retention, whereas a high "Vromann Effect" accompanies fibrinogen replacement and with that has lower impact on the reaction of platelets. Clinically, this does not carry much of importance in connection with the development of thrombus on artificial surfaces, which has to be seen in the light of interactions between fibrinogen and leucocytes.

The adsorption of gamma - globulin (IgG) on artificial surfaces promotes adhesion of platelets and stimulates the reaction to release platelets. The adsorption of gamma - globulin (IgG) follows the adhesion of leucocytes.

Albumin gets rapidly adsorbed on artificial surfaces, despite the fact that it is not one of the main components of protein taking part in the interaction. Adsorbed albumin has the capacity to weaken the adherence of platelets and leucocytes, thereby inhibiting the formation of thrombus. The production of surfaces of this type shows higher blood compatibility. Types of proteins

other than the ones mentioned so far can be in traces and can play a physiological role in the interactions. Adsorbed proteins identified: fibronectin (has a possible influence on adherence of leucocytes), lipoproteins, IgA, IgM, IgD of Willebrand factor XIII multi-protein complex. The latter is a possible connecting link with stronger platelet adhesion and plasminogen with a possible effect on surface - associated fibrinolytic activity. After exposure to blood, the protein coated surfaces guide the intrinsic coagulation through the contact activation phase incorporating the contact protein factor XII (Hagemann factor), factor XI, high molecular weight kininogen (HMWK), and prekallikrein. A cascade of enzymatic reactions result from the adsorption of HMWK and factor XII. Complexed HMWK forms a complex with prekallikrein. This complex splits the factor XII giving factor XII a, which converts the prekallikrein back to kallikrein, which in turn further participates in the formation of HMWK complex. The cyclic reaction, in which kallikrein activates factor XII also ensures the instant availability of kallikrein close to the artificial surface. HMWK is also able to form a complex with factor XI, so that the factor XI required for factor XII can be obtained and the cascade of coagulation can take its course.

2. Reaction of platelets at the artificial surfaces

During interactions of blood with the biomaterial, the blood contact inevitably leads to adhesion and platelet aggregation. The extent to which this takes place is strongly dependent upon the nature of the adsorbed coat of proteins. In compliance with the collagen - induced platelet reaction, it is known that platelet - interaction with adsorbed fibrinogen or gamma - globulin can help in the formation of a complex, which is biochemical reaction between glycosyltransferase of the platelet membrane and incomplete heterosaccharides of the fibrinogen or gamma - globulin. For this reason, the inhibition of platelet adhesion by adsorbed albumin in the absence of saccharide bridges appears to be similar.

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The adherence of circulating platelets to the surfaces covering the proteins leads to a change in the shape of the platelets and from non - nucleated discs to non - nucleated spherocytes with long thread - shaped pseudopodia (viscous metamorphosis). Increasing adhesion of platelets to the irregular coatings of the artificial surfaces gives rise to a growing cross - linking of

marginations of leucocytes with erythrocytes with fibrin. After the adhesion of platelets one can expect the reaction for release of platelets to take place within the adherent platelets with subsequent aggregation on the surface. With regard to their release, the following components of platelets come under the influence of the surface of the membrane. Release of serotonin (5 OH - tryptamine) is less dependant on the actual adherence of platelets and more on the hydrophilic surface properties of the biomembrane. Beta - thromboglobulin (beta - T - G) is released in - vitro through the surface properties and the level of beta - T - G and the platelet factor 4 (PF 4) is found to be higher after implantation of cardiac valves during haemodialysis. Thromboxanes (TXB 2) are reported to be released during intra - aortal balloon angioplasty. Higher level of TBX - 2 is observed during aorticopulmonary bypass. The process of blood coagulation onto an artificial surface indicates the probability of interaction between the platelets and the intrinsic coagulation of blood. The intrinsic coagulation may either be induced by thromboplastins released from the platelets or caused by factor XII - activation by means of the ADP released from the platelets. Formation of thrombin resulting from the intrinsic activation, leads to a rapid generation of monolayer films of fibrin on the artificial surfaces and also leads to preferential treatment being given to adhesion and aggregation of platelets. Production of thrombins

may likewise activate the reaction to release platelets along with secretion of PF4, TXB2 and thrombospondin. It is likely that thrombospondin plays a major role in the aggregation of platelets on the artificial surface. The response of platelets in the context of blood - biomaterial interface is influenced by diffusion and shearing forces.

3. Erythrocytes

When blood makes contact with an artificial surface the erythrocytes also accumulate on the adsorbed protein layers and under certain conditions, a haematolysis occurs in conjunction with the reaction releasing platelets by means of the already - released ADP and fragments of erythrocytes. On adding erythrocytes to protein solutions, a reduction is seen in the quantity of proteins adsorbed. This "red - call effect" is ascribed to the surface contacts of erythrocytes resulting in accumulation of components of membrane and consequently a new, less absorptive surface. Since then, similar reductions in adsorption have been achieved, for instance with the help of cell fragments primarily applied to membranes or particles, although a possible effect results from the competitive adsorption of the hemoglobin released. Absorption of hemoglobin becomes probable with higher surface activity. The clinical use of membrane

oxygenators and the formation of thrombus on the artificial surfaces in the case of artificial heart are promoted by haemodynamics of red cells. Clinical usage of oxygenators is as follows: In cases with an artificial heart, formation of thrombus on the artificial surfaces is promoted by haemodynamics of red blood cells and also by reduced adsorption of protective factors or accumulation of an adhesive substance. Haemolysis is promoted by shearing forces, when the coagulation is induced under negligible shearing force, so that the erythrocytes and fibrin together form the red thrombus.

4. Leucocytes

The adhesion of leucocytes to an artificial surface has been known for a considerable period of time. The adhesive power of PMN - leucocytes was higher compared to that of the lymphocytes (examination of leukapheresis). The adhesion of leucocytes is also influenced by the adsorbed monolayer protein coats. Surfaces that were coated with IgG, thrombin and prothrombin, showed an increased leucocytic adherence. With some reservations about the sensitivity of leucocytes in a mechanical alteration, behavior of leucocytes accumulation on artificial surfaces is similar to that of blood platelets. Shear forces influence the aggregation of leucocytes and the incorporation of leucocytes in the micro-

aggregation platelets. Formation of thrombin on artificial surfaces is in contrast with the relatively passive behavior of erythrocytes as against the action of leucocytes. Leucocytes are adsorbed by the thrombus and incorporated in the thrombotic process. Leucocytes influence the recruitment of platelets with the help of a lyosomal enzyme so that they can instantly take part in the fibrinolysis.

The direct role played by the leucocytes in the formation of thrombus on artificial surfaces is a result of adhesion of leucocytes and the effect of this adhesion on aggregation of platelets. This process develops from the bond of endogenic pro - coagulative, pro - aggregation activities. The interactions between leucocytes and the artificial surfaces weaken the immunophagocytosis with reduced resistance to infection. The adhesion of leucocytes leads to a growth in proteins in the context of specific cell functions. This results in the release of superoxide radicals, leucotriene, interleukin, Tumor - necrosis factor, plasminogen activator, prostaglandins, histamines, and platelet activation factor. A part of the interactions refers to the activation of the complement, which is discussed in the next point.

5. Activation of complement

In haemodialysis, the influence of artificial surfaces on activation of complement can be observed with regenerated cellulose membranes. It is noticeable that the C3 molecule is split and the classical reaction progresses through C1 - C2 - C4 molecules. The alternative way shows that the C3 molecule does not involve the earlier complement - components. The fact that polysaccharides play the role of activators in the alternative way, seems to be in line with the observation that cellulose membranes can activate the reaction this way (the alternative way) (Cardio - pulmonary bypass operation). As against this, studies on leukapheresis show that artificial surfaces activate the common pathway. The following complement - components are of interest: Anaphylatoxin molecule C3a, C4a, C5a. These function as inflammatory mediators. The clinical implication of complement activation

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lies in enabling adhesion of leucocytes and platelets to the polymeric surfaces.

6. Fibrinolytic activity (compare diagram 3)

7. Objectives and variations of the test parameters

The objective of the invention is to modify the surface of the implant in order to improve blood compatibility such that the conditions of diffusion for capsulated cells of the islets (see introduction) are optimal. The improvement in compatibility is achieved by:

1. Changing the physical properties of the surface (such as improvement in texture of rough surfaces)
2. Reinforcing the hydrophilic properties.

Hydrophilic surfaces reduce not only protein adsorption but also cellular adsorption. Most of the hydrophilic surfaces are polymeric hydrogels having only limited mechanical consistency (swelling in aqueous solution). This can be improved by coating or by copolymerization. Hydroxy ethyl methacrylate (HEMA) is substituted by polyethylene oxide (PEO) because it has a lower complement activation compared to HEMA. A modification of polymeric surfaces is the concept of partial transfer of charge by complexing on conjugated polymers (Literature: Ulmann's encyclopedia of industrial Chemistry, volume A 21, pages 429 to 445 (1992), Verlag Chemie, Weinheim).

Principle

Addition of electron donators or acceptors during reduction or oxidation results in a change in the basic properties of a

polymer. This imparts metallic conductivity to the polymeric framework, originally being insulative in nature. This redox reaction helps to achieve a distinct increase in the motion of electrons and consequently a high electrical conductivity. To achieve this electrical conductivity, it is particularly necessary that the partial charge be transferred from the donor to the acceptor, in addition to the high atomic number of the charge transfer partner.

The objective is to make the hydrophilic / hydrophobic behavior auto-regulatory as may be required by suitably modifying or introducing side-groups. This would mean that the decisive interactions like the adherence of proteins in monolayer form can be counteracted by the consecutively involved mechanisms of thrombogenicity such as platelet activation and adherence, intrinsic activation of coagulation, adherence of leucocytes etc. through the artificial surface.

Variations in the test parameters

1. Permeability

Polymer films of various types:

- polyamide
- polyester
- polyolefins

- polysilicones
- polystyrene
- copolymerization
- polyflour

thickness, gas pressure, O_2 / N_2 / CO_2, moisture, H_2O, isotonic liquids, blood among others

2. O_2 - content in blood using ESR, NMR

Radicals in blood

arterial, venous

3. Supplementary properties of the permeability of polymers reinforced with electrically conductive materials like lampblack or other conductive partners, intrinsically conductive polymers such as PPY.

4. Surface behavior

O_2

a) reversible e.g. pump off
b) fixed
c) chemically reacted: peroxide, hydroxide, COOH (carboxylic acid), CHO (aldehyde), OH (alcohol), e.g. using ESCA, IR or the like.

d) Time, pressure, conditions including physiological.

5. Polymer surfaces coated with oxygen

(or air, nitrogen, carbon dioxide, carbon monoxide)

Control check using ELF

Surface: dry, moist (i.e. special solvent systems)

6. Agent - release

a) Correlation: Carrier material

electrically conductive polymers

neutral materials

(indifferent)

b) Type of agent (e.g. insulin), various types of preparations, extent of release.

6. Polymer depot materials: variations in their types and coatings

Double experiments carried out in - vivo and in - vitro to observe the functions of the Langerhans' islet cells in silicon catheters.

Introduction: From the point of view of physical flow, the rapid progression of functional immobilization of capsulated islet

cells in the vascularization model with a connection to the blood circulation (circular capillaries with open pores in textile carrier of a vascular endoprosthesis), stems from a laminar flow profile and the dynamic viscosity helps the adherence of molecular structures. As against the vascular system of the circulation, we do not have any such symmetry of a laminar velocity profile in the heart to stop the adherence of proteins. For this reason, the right ventricle of the heart is suited for implanting capsulated tissues with simple peripheral access as well as for physical flow.

Material and method: In this method, the immunological aspects of immuno-separation membranes are not taken into consideration. As a matter of ethical responsibility

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towards trials conducted on grown animals, we have used a model, in which an in - vitro and an in - vivo statement is coupled together. By doing so, the generation of an irreversible disease during the experiment, can be avoided, without affecting the in - vitro part of the experiment qualitatively.

Following is the procedure: We have implanted 3000 islet cells of lewis rats in two microporotic silicon catheters with a molecular

cut - off between 50 and 140 kilo - Dalton and a capillary diameter between 500 and 600 micron.

Isolation and preparation: The islet cells in the case of lewis rats were isolated by ductal enlargement (10 ml Krebs - Ringer solution and a 2 - step collagenase digestion (1.2 and 0.7 mg / ml sigma type XI, St. Louis, USA, van Strylichem et. al)). The degree of purity was achieved by discontinuous dextran - gradients. Prior to transplantation, the cells were incubated overnight with RPMI - 1640 medium and 10% fetal bovine serum (Gibco, Karlsruhe)

The organic cells were then filled into the capillary membrane system of the silicon catheter. Immediately thereafter, the first catheter was implanted via the external jugular vein in the ventricular system of a non-diabetic sheepdog. After fixing it externally, the catheter dwells in the organism for about 4 weeks. The second catheter was placed extravascular under the abdominal wall. To charge glucose statically, we divided the catheter into six small segments and equilibrated with 50 mg of % glucose in a petri - dish for a period of 60 minutes (in 5 ml Krebs - Ringer buffer pH 7.4% RIA grade bovine albumin sigma, Heidelberg). After an hour, the islet cells were stimulated with 300 mg % glucose for two hours. To judge the capacity of insulin secretion to down-regulate after stimulation, the islet cells were finally poured into 50% glucose medium. This medium was

replaced after 30, 60, 180 and 210 minutes. After 30, 60, 180, 210 and 240 minutes, test samples were taken for measurement of insulin. The concentration of insulin was determined with the help of a radioimmunoassay (RIA Gnost Behring Werke, Marburg) (Standard value of insulin in rats, Novo Koepenhavn). Each value represents two values.

Results: With 50% glucose charge, the value of insulin secretion after 30 minutes measured 250 in U/I. No insulin could be proven to be secreted from the extravascular control segment (catheter in the abdominal wall). The value after 60 minutes read 250 in U/I. With 300 mg % glucose charge, the reading after two hours measured 750 in U/I (Check: no insulin released). The value after 180 minutes was observed to be 1600 in U/I, which happened to be the highest value (Check was negative). The insulin secretion after four hours was lowered to 200 in U/I (down-regulation).

Discussion: After a static charge of glucose, the in - vitro values of insulin measured in a non - diabetic sheepdog having silicon catheters implanted with capsulated islet cells are seen to dwell for four weeks and this is an evidence of sufficient nutrition or oxygenation in a venous compartment. For this reason, the flow may be seen as a major factor hindering adhesion. At the same time, the functional failure in the extravascular control system is adequate to provide evidence to the contrary.

Patent Claims

1. Immobilized organic material to release a definite quantity of agent is characterized by the fact that the material described in the invention can be implanted in - vivo in human beings.
2. As per claim 1 of the Patent, the product is characterized by the fact that the implant is placed or introduced in the nerve tracts of the receiving organism.
3. As per claims 1 and 2 of the Patent, the product is characterized by the fact that insulin, proinsulin, preproinsulin and / or organic cells (islets of Langerhans, APVD - amine precursor uptake decarboxylating)systems of xenogenic or autogenic origin can be used as organic immobilized materials.
4. As per claims 1, 2 and 3 of the Patent, the product is characterized by the fact that the immobilization of the organic material takes place with the help of synthetic and / or natural high molecular weight compounds.
5. As per claims 1, 2, 3 and 4 of the Patent, the product is characterized by the fact that the immobilization system contains definite polar groups, which on application of electric voltage, allow release of a definite quantity of

the agent.

6. As per claims 1, 2, 3, 4 and 5 of the Patent, the product is characterized by the fact that the immobilization system contains components, which either suppress or prevent agglomeration of the blood.
7. As per claim 6 of the Patent, the product is characterized by the fact that Heparin, Hirudin, Marcumar or their derivatives and / or modifications are used to antagonize agglomeration.
8. As per claims 1, 2, 3, 4, 5, 6 and 7 of the Patent, the product is characterized by the fact that the immobilization system consists of a porous or a hollow material.
9. As per claim 8 of the Patent, the product is characterized by the fact that the immobilization system consists of a polymeric material with a larger internal or external surface.
10. As per claim 9 of the Patent, the product is characterized by the fact that hollow bodies like hoses, tubes, carbon fibers, or microcapsules or even sponges, foams with open pores and / or films, woven fabric and / or non-woven fabric with micro pores or larger surfaces are used as immobilization systems.
11. As per claims 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 of the

Patent, the product is characterized by the fact that the release of agent is timely and need - based.

12. As per claims 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11 of the Patent, the product is characterized by the fact that a long term antagonization of blood components adhering to the membrane and the mutual interchange of agent between the donor tissue and the receiver is guaranteed for the open - pored immuno - separation immobilization system described in the invention.

This is supplemented by three pages of drawings

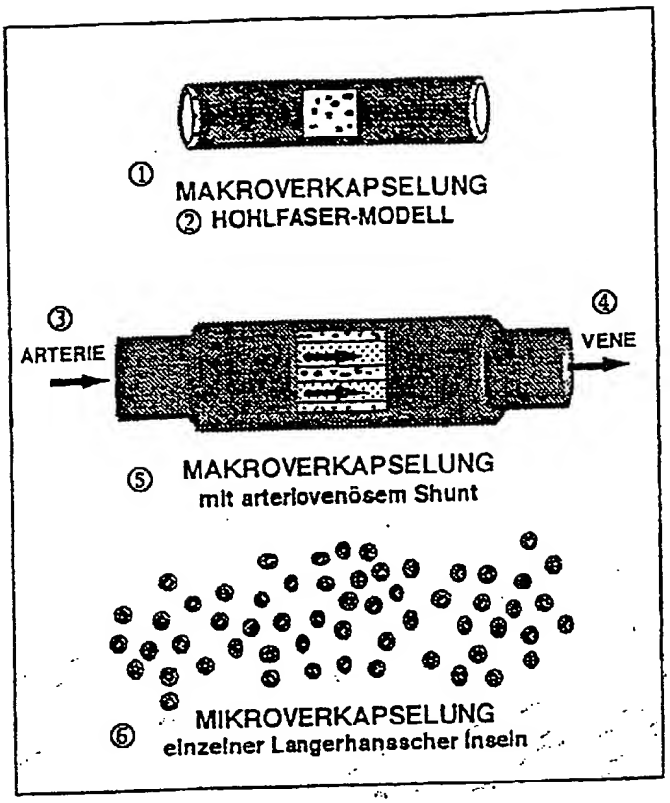

Diagram 1

Schematic representation of different implantable models of a biortificial endocrine pancreas

Key:

1) Macro-capsulation, 2) Hollow chamfer model, 3) artery, 4) vein, 5) Macro-capsulation with arteriovenous shunt, 6) Macro-capsulation of individual Langerhans' islet cells

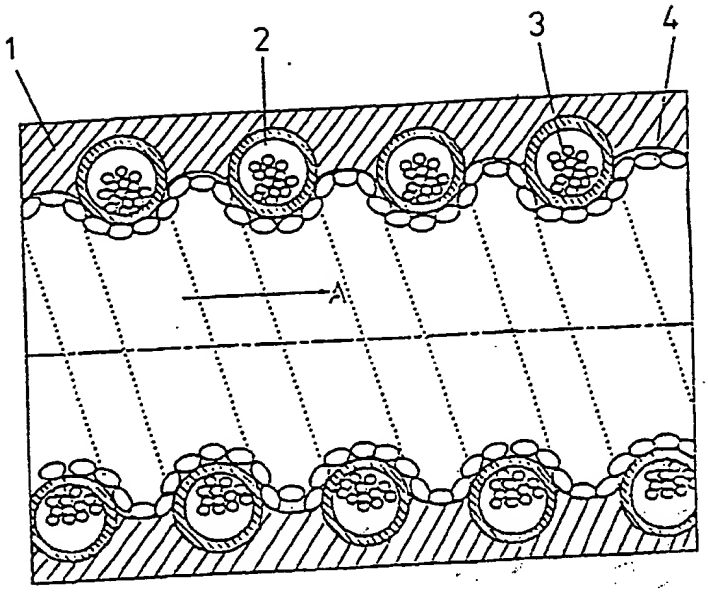

Diagram 2

Schematic cross section of the macro-device (Vascular prosthesis with integrated capillary membrane). 1) Vascular prosthesis made of PU (polyurethane) - fleece, 2) Capillary membrane, 3)Langerhans' islet cells, 4) growing endothelial layer of cells, A) Direction of flow of blood.

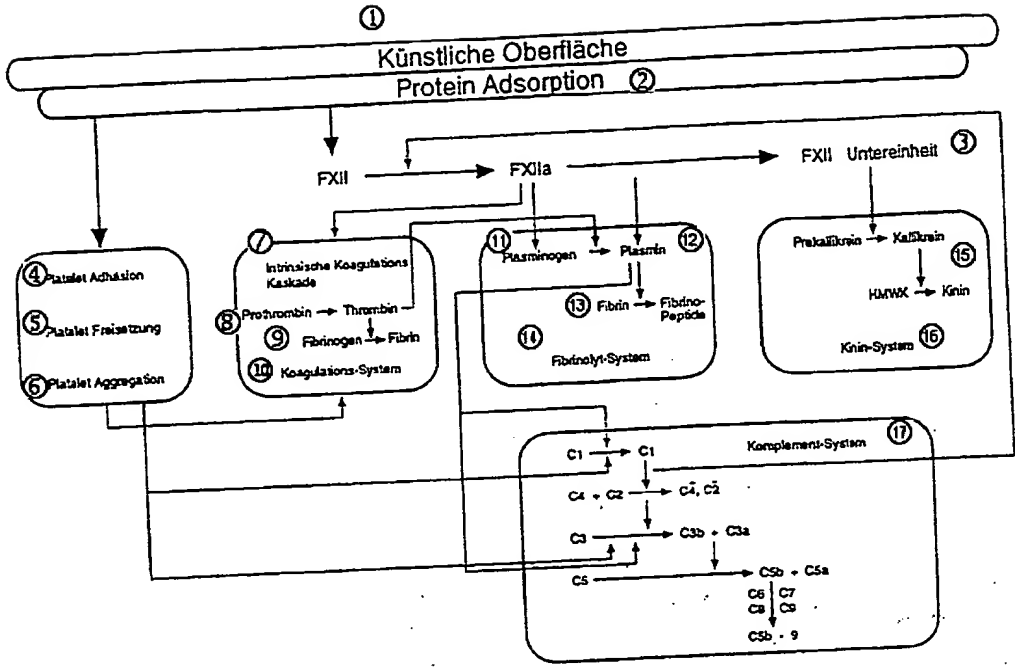

Diagram 3 Reaction of blood with an artificial surface

Key:

1) Artificial surface, 2) Protein adsorption, 3) Subunit, 4) Adhesion of platelets, 5) Release of platelets, 6) Aggregation of platelets, 7) Cascade of intrinsic coagulation, 8) Prothrombin → Thrombin, 9) Fibrinogen → Fibrin, 10) Coagulation system, 11) Plasminogen, 12) Plasmin, 13) Fibrin → Fibrinopeptides, 14) Fibrinolytic system, 15) Prekallikrein → Kallikrein, 16) Kinin system, 17) Complement system